Does the distribution pattern of brain metastases during BRAF inhibitor therapy reflect phenotype switching?

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Abstract: Brain metastases (brain mets) are frequent in metastatic melanoma patients. The aim of this study was to investigate the morphology and progression pattern of brain mets in melanoma patients treated with BRAF inhibitors (BRAFi) compared with patients who did not receive targeted therapy (BRAFi group and control group). The number and size of brain mets were compared between a baseline and a comparative MRI at progression. The number of brain mets was grouped into seven number classes (N=1-4, N=5-10, N=11-20, N=21-30, N=31-40, N=41-50, and N>50) and its difference was reported as the change of class that occurred. The mean size of the newly developed lesions was determined by representative measurements and the evolution of three persisting target lesions was assessed on the basis of modified RECIST criteria. Of 96 patients studied, 42 were in the BRAFi group and 54 were in the control group. Patients under BRAFi treatment had a significantly greater increase in the number of brain mets, where the median change of class for the BRAFi compared with the control group was 2 versus 0 (P<0.01). The mean size of the new lesions was smaller in the BRAFi group. Pre-existing target lesions did not show any prominent or different patterns of how they evolved in either group. Brain mets in patients treated with BRAFi showed a progression pattern characterized by a high propensity to disseminate, which might reflect an in-vivo manifestation of phenotype switching in response to targeted therapy, with a predominance of the invasive/migratory tumor cell phenotype. Drivers of invasiveness may present promising targets for therapeutic interventions.

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Keywords: BRAF inhibitor, brain metastases, melanoma, phenotype switch, progression pattern, targeted therapy

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Introduction

Melanoma has a high propensity to metastasize to the brain. Up to 20% of melanoma patients present with brain metastases (brain mets) at diagnosis of stage IV and ~40–50% ultimately develop them during the course of their disease [1,2]. Overall, central nervous system involvement heralds a poor prognosis [3,4].

The introduction of immunotherapy and targeted therapy has changed the standard of care in the treatment of advanced melanoma. On the basis of the discovery that 40–60% of melanomas harbor a targetable BRAF V600 mutation, a new era of mitogen-activated protein kinase inhibitors (MAPKi) has been established [5]. Selectively targeted BRAF-inhibitors (BRAFi, e.g. vemurafenib, dabrafenib) have shown impressive clinical efficacy and have led to improved survival in patients with V600-mutated metastatic melanoma [6,7]. Subsequently, the superiority of concurrent BRAF/MEK inhibition (e.g. vemurafenib/cobimetinib, dabrafenib/trametinib) has been reported in several clinical trials [8,9]. In patients with brain mets, evidence of clinical activity of BRAFi is emerging [10,11], and currently available pooled data show a median overall survival of 7.9 months in this subset of patients [12]. Although a growing body of research is evaluating the efficacy and objective response rates of MAPKi to further determine their role in patients with brain mets (NCT01378975, NCT02230306, NCT02039947), the progression pattern in the brain and the influence of targeted therapies on this process have not been investigated very extensively so far. Therefore, the aim of this current study is to analyze the morphology and pattern of brain mets among melanoma patients treated with BRAFi and assess potential differences compared with patients who did not receive targeted therapies.

Patients and methods

Patient selection and data acquisition

This single-center retrospective analysis was carried out at the Department of Dermatology of the University

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Hospital Zurich. Patients treated at our institution for stage IV melanoma with brain mets were identified from our Melanoma Registry, which records melanoma patients since 2008, and through our interdisciplinary oncology board meetings. Eligible patients were 18 years of age or older, had histologically confirmed stage IV cutaneous or mucosal melanoma, and at least one intracranial tumor lesion. The study protocol was approved by the local ethics committee (EK647/KEK-ZH-Nr. 2014–0193).

Patients were divided into two groups on the basis of whether (BRAFi group) or not (control group) they received targeted therapy. Targeted therapy included BRAFi monotherapy and/or BRAFi/MEKi combination therapy. Electronic medical records were reviewed for demographical and clinicopathological parameters. Data extraction included age, sex, primary tumor characteristics, mutational status, and details of systemic and locoregional brain therapies.

Definition of baseline and comparative study

To analyze the change in pattern in brain mets in patients exposed to BRAFi versus those not exposed, two contrast-enhanced MRI studies of the brain were compared. The baseline (t_1) and comparative image (t_2) for the two different groups were defined as follows: in the BRAFi group, brain mets evolution under treatment exposure to targeted therapy was examined. Accordingly, the last MRI obtained immediately before treatment initiation with BRAFi (baseline image) was compared with the last MRI before termination of BRAFi treatment (comparative image). In the control group, each patient's initial and terminal brain mets status was examined. This was illustrated by the MRI of first radiographical brain mets detection (baseline image) and the last available brain MRI in a patient's disease course (comparative image).

Table 1 Patient baseline characteristics

	BRAFi group	Control group
Total number of patients	42 (44)	54 (56)
Age [median (range)]	54 (24–73)	65 (35–86)
Sex		
Male	25 (60)	36 (67)
Female	17 (40)	18 (33)
BRAF status		
Mutated	42 (100)	6 (11)
Wild type		33 (61)
Unknown		15 (28)
BRAF genotype		
V600E	27 (64)	4
V600K	10 (24)	
Unknown	4 (10)	1
Other[a]	1 (2)	1
Localization of primary		
Head and neck	9 (21)	10 (19)
Trunk	13 (31)	19 (35)
Upper extremity	7 (17)	7 (13)
Lower extremity	6 (14)	11 (20)
Unknown	7 (17)	7 (13)
Histological subtype		
Nodular	15 (36)	14 (26)
Superficial spreading	6 (14)	5 (9)
Acrolentiginous		4 (7)
Lentigo maligna		7 (13)
Mucosal	1 (2)	1 (2)
Other	8 (19)	9 (17)
Unknown	12 (29)	14 (26)
Breslow index (mm)		
0.1–1.0	5 (12)	12 (22)
1.01–2.0	9 (21)	9 (17)
2.01–4.0	9 (21)	12 (22)
>4.0	7 (17)	10 (19)
Unknown	12 (29)	11 (20)

Age is reported in years as median age and range. All other variables are reported in *n* (%).
[a]K601E, D594H.

Table 2 Type of systemic therapies received before the evaluation period for the BRAF inhibitor and the control group

Systemic therapies before t_1	BRAFi group ($N=42$)	Control group ($N=54$)
No systemic therapy	32 (76)	34 (63)
Chemo only	7 (17)	7 (13)
Immuno only	2 (5)	4 (7)
Chemo + immuno		6 (11)
Chemo + mki		2 (4)
Chemo + immuno + mki	1 (2)	1 (2)

Variables are reported in *n* (%).
Immunotherapy included ipilimumab, nivolumab, pembrolizumab, and tremeliumab. Multikinase inhibitor therapy included sorafenib, pazopanib, and imatinib.
BRAFi, BRAF inhibitor; Chemo, chemotherapy; Immuno, immunotherapy; mki, multikinase inhibitor therapy.

Pattern analysis: number and size of brain mets

A semiquantitative analysis of the morphological change of brain mets in terms of the number and size was carried out. The number of brain mets was stratified into seven number classes ($N=1$–4, $N=5$–10, $N=11$–20, $N=21$–30, $N=31$–40, $N=41$–50, and $N>50$ metastases). The difference in the number of metastases between the baseline and the comparative image was reported by the change of class that occurred. Possible changes therefore were a reduction of class (−), no change of class (=), and an increase of 1–6 (+ to +++++++) brain mets number classes.

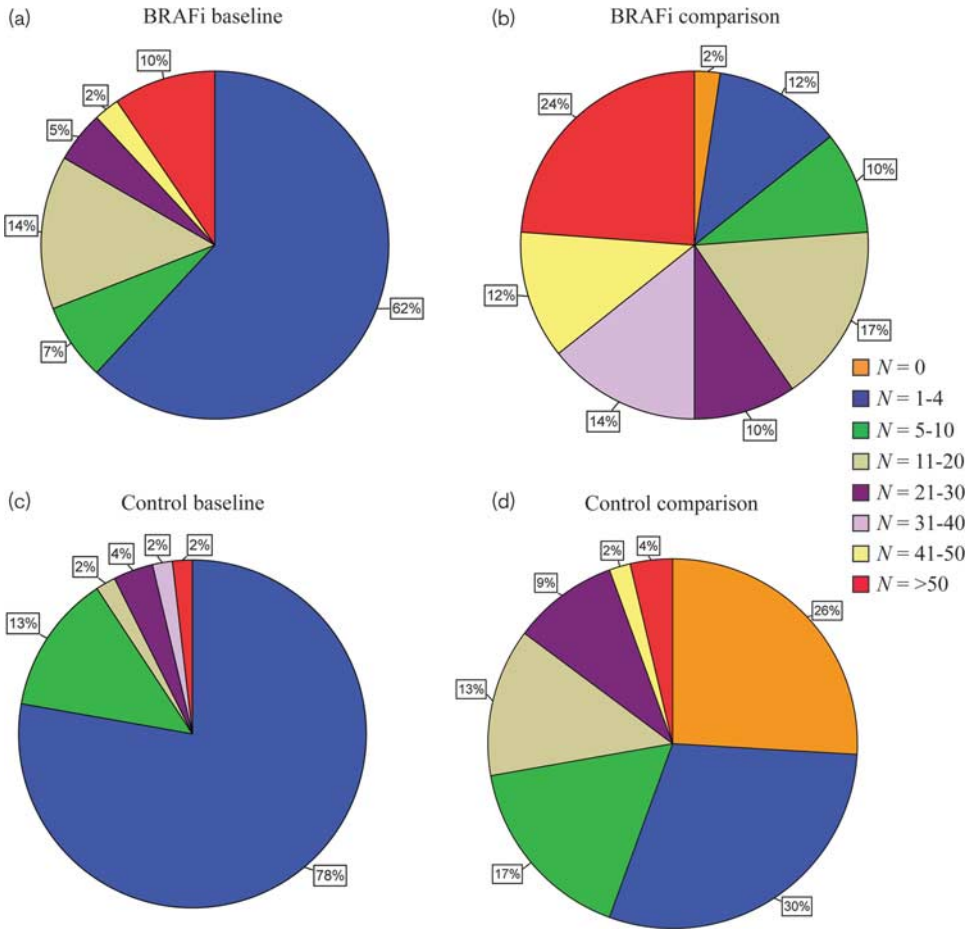
To investigate how persisting lesions evolved during the evaluation period, up to three target lesions present and

Table 3 Locoregional brain therapies administered to the BRAF inhibitor and the control group during the evaluation period

Brain therapies during t_1–t_2	BRAFi group ($N=42$)	Control group ($N=54$)
No brain therapy	24 (57)	7 (13)
Surgical resection	2 (5)	1 (2)
Radiation therapy (WBRT, SRS or both)	13 (31)	22 (41)
Combination of resection and radiation	3 (7)	24 (44)

Variables are reported in *n* (%).
t_1–t_2 represents the evaluation period between the baseline and the comparative image.
BRAFi, BRAF inhibitor; SRS, stereotactic radiosurgery; WBRT, whole-brain radiation therapy.

Fig. 1



Number of brain mets. These pie charts show the relative distribution and longitudinal evolution of the number of brain mets (categorized in number classes) for both patients who were treated with BRAF inhibitors (BRAFi group) and those who did not receive targeted therapy (control group). *N*, number of brain metastases. (a) Number class at baseline, BRAFi group. (b) Number class at comparison, BRAFi group. (c) Number class at baseline, control group. (d) Number class at comparison, control group. Brain mets, brain metastases.

reproducibly measurable in both baseline and comparative images were defined, and their largest sum of diameter was measured. The difference in the largest sum of diameter was calculated and then reported as stable disease, progressive disease, partial response, or complete response according to a modified application of the RECIST 1.1 criteria. An estimate of the mean size of the newly developed brain mets at t_2 was made by measuring up to five lesions considered to be representative of the new lesion's predominant size. All analyses were carried out by the same investigator.

Statistical analysis

For statistical analysis, Microsoft Excel 2011 (version 14; Microsoft Corporation, Redmond, Washington, USA) and SPSS (version 22; IBM, Armonk, New York, USA) software was used. Descriptive results were reported as numbers, percentages, mean, and median. Data were analyzed using the Mann–Whitney *U*-test and the Student *t*-test. A *P*-value below 0.05 was considered to indicate statistical significance.

Results

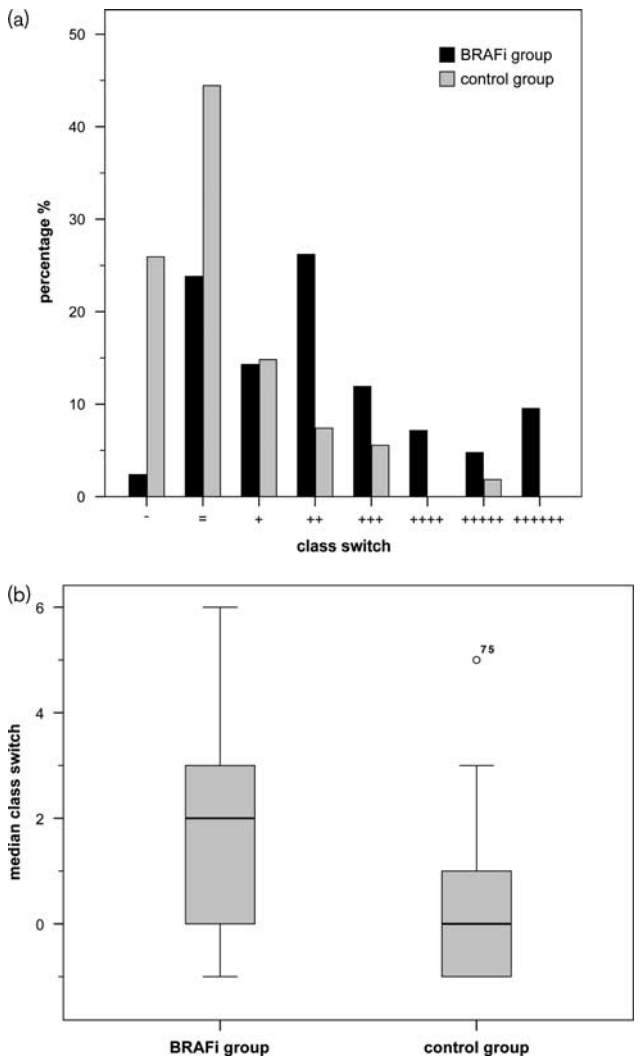
Patient, disease, and treatment characteristics

A total of 96 patients were included for analysis and patient baseline characteristics are reported in Table 1.

In the BRAFi group, 32 (76%) patients received targeted therapy upfront, whereas the remaining 24% had other systemic therapies before BRAFi treatment. In the control group, 20 (37%) patients received systemic therapies before the evaluation period. Details on the systemic therapies patients received before the evaluation period are shown for each group in Table 2.

During the evaluation period, patients in the BRAFi group received either BRAFi monotherapy, in 83% (all vemurafenib, $N=35$), combination therapy, in 7% (vemurafenib/cobimetinib, $N=1$, encorafenib/binimetinib, $N=2$), or monotherapy

Fig. 2



Brain mets number class switch. (a) The longitudinal change in number of brain mets grouped into classes is shown with the respective percentages for both the BRAFi and the control group.'−': reduction of class.'=': no change in class.'+' to '++++++': increase in 1–6 brain mets number classes. (b) Median brain mets number class switch between the baseline and comparative image for the BRAFi and the control group: Boxplot, $P<0.01$. BRAFi, BRAF inhibitors; Brain mets, brain metastases.

with vemurafenib, followed by combination therapy, in 10% (vemurafenib/cobimetinib, $N=2$, dabrafenib/trametinib, $N=2$). Patients in the control group received systemic therapy, 89%, during the evaluation period (chemotherapy alone, $N=21$, immunotherapy alone, $N=13$, multikinase inhibitor alone, $N=1$, chemotherapy and immunotherapy, $N=7$, chemotherapy and multikinase inhibitor, $N=6$).

Locoregional brain therapies including surgical resection, radiation therapy (stereotactic radiosurgery and/or whole-brain radiation therapy), or a combination of both surgical resection and radiation therapy were more frequently used in the control group than in the BRAFi group (87 and 43%, respectively). Details on brain therapies administered during t_1 and t_2 are shown for each group in Table 3.

The median observation time between the baseline and comparative image (t_1–t_2) was similar in both groups, with 5.8 (range: 1.6–20.7) months in the BRAFi group and 5.3 (range: 0.6–38.2) months in the control group. The median treatment duration with targeted therapy in the BRAFi group was 5.8 (range: 1.9–24.4) months.

Number of brain mets

The majority of patients in both the BRAFi and control groups had 1–4 brain mets at baseline (62 and 78%, respectively). More patients in the BRAFi group already had more than 10 brain mets at baseline (31 vs. 9%). The distributions of brain mets present at baseline and comparative images are shown for each group in Fig. 1.

In the comparative MRI, there was a significantly higher increase in the number of brain mets in the BRAFi compared with the control group ($P<0.01$). The majority (60%) of the patients in the BRAFi group showed an increase of more than one number class, whereas only 15% in the control group showed an increase of more than one number class (Fig. 2a). The patients in the BRAFi group showed a median increase of two number classes, whereas patients in the control group remained in the same class (median class switch of 2 vs. 0, $P<0.01$, Fig. 2b).

No change in class or even a reduction in class was more frequently observed in the control group than in the BRAFi group (44 and 26% vs. 23 and 2%, respectively).

Size of brain mets

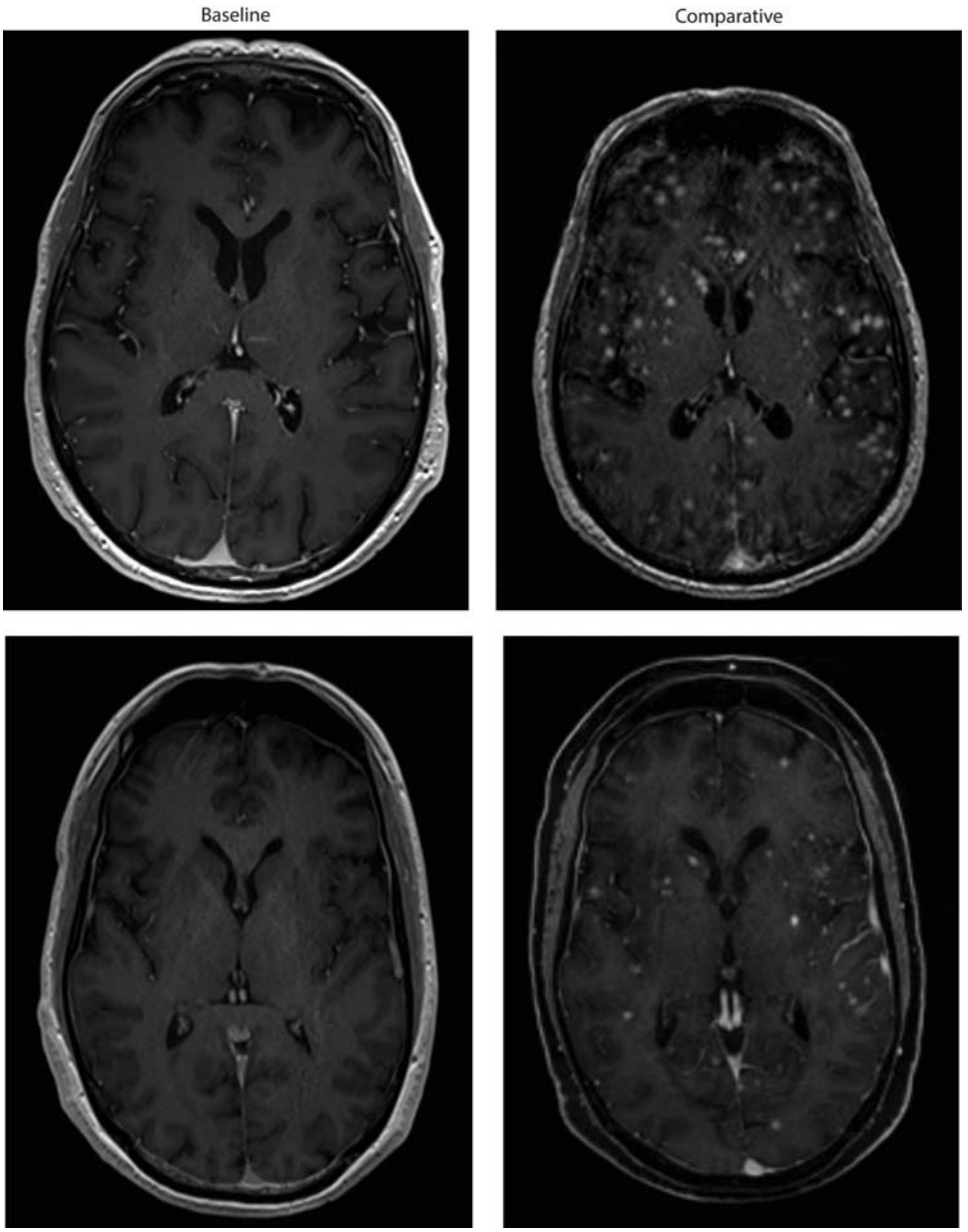
Pre-existing target lesions did not show any prominent or different patterns of how they evolved in either group. A higher percentage of patients in the BRAFi group showed a partial response of their target lesions (29 vs. 13%). Fourteen (26%) patients in the control group showed a complete response associated with a reduction of class, but only one patient in the BRAFi group.

New emerging lesions measured at t_2 were in general smaller in the BRAFi group (mean size 5.9 mm) compared with the control group (mean size 7.4 mm). This difference was not statistically significant ($P=0.15$).

Discussion

BRAF-targeted therapies have become a very important component in the treatment of patients with BRAF-mutated metastatic melanoma, including patients with brain mets. To our knowledge, this is the first study to focus on the morphology and pattern of brain mets progression in melanoma patients treated with BRAFi compared with patients not exposed to such therapies.

Fig. 3



Brain metastasis (brain mets) evolution on MRI. Axial t_1-weighted gadolinium-enhanced MRIs of the brain of two BRAF mutant melanoma patients treated with a selective BRAF inhibitor (exemplary for the BRAFi group) showing a significant increase in the number of brain mets between the baseline (left) and the comparative image (right). Each patient is shown as a horizontal line.

Our data indicated that patients under BRAFi frequently showed a marked increase in the number of brain mets during targeted therapy. MRIs at the time of comparison often showed more multiple and smaller brain lesions than before initiation of BRAFi treatment at baseline and, in some cases, showed innumerable contrast-enhancing punctate lesions (Fig. 3), indicative of a miliary, disseminated distribution pattern of brain mets. In the control group, such intracranial disease courses were far less likely to occur and the majority of patients did not even experience an increase in the number class of present brain mets at all.

These results are intriguing because they suggest an unusual dissemination pattern of brain mets under BRAFi treatment and consequently, the question arises whether this pattern is unique to the brain or might reflect a general effect of BRAFi treatment. Azer *et al.* [13] investigated patterns of response and progression assessed by disease-site (intracranial vs. extracranial) in

23 patients with melanoma brain mets treated with dabrafenib. They concluded that there was no dominant site (intracranial vs. extracranial) or pattern (new vs. existing lesions) of disease progression and site-specific response was concordant in 71% [13]. In contrast, Seifert *et al.* [14] found in a retrospective study that response to vemurafenib was indeed dependent on the anatomical site and complete or partial response was particularly low in the brain. In addition, the brain was the most common site for new metastases to develop [14]. Similarly, there was considerable remission of extracerebral disease, but intracranial metastases developed or progressed in a series of patients [15].

Recently, many researchers have intensively investigated the various mechanisms driving tumor progression and drug resistance in melanoma. One model that has been able to link the changes observed in cellular behavior and molecular biology during metastatic progression and has increasingly been invoked is the phenotype switching model. It is based on the characterization of different gene expression signatures that define two populations of melanoma cells, distinctive by showing either a more invasive or a proliferative phenotype [16,17]. These transcriptional states are interchangeable programs between which melanoma cells switch back and forth, catalyzing repeated episodes of dissemination and tumorigenesis, where the dynamics of this process are markedly influenced by local microenvironmental factors [17,18]. The transition from a proliferative to an invasive phenotype shares many features of epithelial-to-mesenchymal transformation and is associated with a characteristic cell morphology switch from predominantly rounded to elongated cells [19–21]. In line with other researchers, we believe that melanoma cells use phenotype switching as an escape mechanism to targeted therapy as it has been observed that proliferative melanoma cells can acquire invasive features upon MAPKi, and as a result, not only become less susceptible to growth inhibition but also have greater ability to metastasize [19,22,23]. Apart from this, there is growing literature stating an increased activation of the AKT-PI3K pathway in melanoma brain mets compared with other distant metastatic sites [15,24], and melanoma cells stimulated by astrocyte-conditioned medium showed higher AKT activation and invasiveness, adding evidence that extrinsic factors in the brain microenvironment may induce these brain-specific molecular alterations [14,15,25]. This supports the hypothesis that the tumor microenvironment differs between the body and the brain and that this type of resistance mechanism to BRAFi could be specific to the brain. Yet, in autopsy studies of some of our BRAF-mutated melanoma patients, we have observed a similar disseminated metastatic spread in other organs than the brain, including the lung and the liver.

There are limitations in this study and its retrospective nature is certainly one of them. Tailored to each patient's individual needs, the two groups are heterogeneous in the systemic and local brain treatments received before and during the evaluation period. Apart from the fact that the group allocation was primarily dependent on the patient's BRAF mutational status, t_1 and t_2 represented logical time points on the basis of exposure to a specific therapy in the BRAFi group, whereas a slightly different timeline was observed in the control group. In addition, the semiquantitative approach by grouping the number of brain mets in classes and reporting class switches instead of exact numbers and differences limits accuracy. Still, we are convinced that our results show clear and reliable differences between the two groups.

Conclusion

Patients under BRAFi showed a distinct pattern of progression in the brain. It seems that BRAFi therapy can facilitate metastatic dissemination in the brain, leading to a miliary pattern of brain mets presenting with multiple small punctate lesions in MRIs. This might reflect an in-vivo manifestation of phenotype switching in melanoma progression and suggests the predominance of an invasive/migratory tumor cell phenotype. Therefore, drivers of invasiveness may present promising targets for therapeutic interventions.

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Conflicts of interest

R.D. receives research funding from Novartis, Merck Sharp & Dhome (MSD), Bristol–Myers Squibb (BMS), Roche, GlaxoSmithKline (GSK), and has a consultant or an advisory board relationship with Novartis, Merck Sharp & Dhome, Bristol–Myers Squibb, Roche, GlaxoSmithKline, and Amgen outside the submitted work. S.M.G. receives travel grant support and is an intermittent board advisory member for Bristol–Myers Squibb, Merck, Novartis, and Roche, and receives research funding from the University of Zurich. And for the remaining authors there are no conflicts of interest.

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